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Your Case No.: BP0270-US3

BV

(Translation)

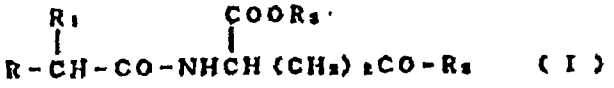
Japanese Laid-Open Publication No. 1-125357

SPECIFICATION

Title of the Invention: TRIPETIDE DERIVATIVES

What is claimed is:

1. A tripeptide derivative of the following formula:

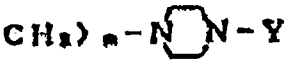


(I)

or a salt thereof;

wherein:

R is hydrogen, lower alkyl or benzyl; R_1 is $-(NH)_m-(CH_2)_n-W$ or



, m is 0 or 1 and n is an integer from 0 to 4, W is hydrogen, carboxyl, amino or hydroxy, Y is hydrogen, lower alkyl, phenyl or benzyl; R_2 is hydrogen or lower alkyl; R_3 is a group of formula selected from

or $-N(R_4)-CH(R_2)-COOR_2$

wherein:

R_4 is C_{4-8} cycloalkyl, or phenyl substituted with halogen , lower alkoxy or hydroxy.

DETAILED DESCRIPTION OF THE INVENTION

The current invention relates to novel tripeptide derivatives and salts thereof and more specifically, to tripeptide derivatives and salts thereof (hereinafter, referred to compounds of the invention) represented by the following formula:

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(I)

or a salt thereof;

wherein:

R is hydrogen, lower alkyl or benzyl; R_1 is $-(NH)_m-(CH_2)_n-W$ or

, m is 0 or 1 and n is an integer from 0 to 4, W is hydrogen, carboxyl, amino or hydroxy, Y is hydrogen, lower alkyl, phenyl or benzyl; R_2 is hydrogen or lower alkyl; R_3 is a group of the formula selected from

or $-N(R_4)-CH(R_2)-COOR_2$

wherein:

R_4 is C_{4-8} cycloalkyl or or phenyl substituted with, halogen, lower alkoxy or hydroxy.

The following compounds are disclosed as Angiotensin Converting Enzyme inhibitors(hereinafter, referred to ACE inhibitors) in Journal of Medicinal Chemistry 28(11), 1606-1611(1985): 1-(L-lysyl-γ-D-glutamyl) indoline-2(S)-carboxylic acid and 1-(N^2,N^6-dibenzyloxycarbonyl-L-lysyl-γ-D-glutamyl) indoline-2(S)-carboxylic acid.

These two compounds are apparently different from the compounds of the invention because the corresponding R group of these compounds is amino or benzyloxycarbonyl which differ structurely the compounds of the invention.

These two compounds are useful, based on ACE inhibitory activity thereof, as therapeutic agents for hypertension, ischemic cardiac insufficiency and other cardiovascular diseases.

As used herein, "lower alkyl" is a linear or branched alkyl group with 1-5 carbon atom(s). Preferable lower alkyl groups include methyl, ethyl and iso-propyl.

"Lower alkoxys" include methoxy, ethoxy, tert-butoxy and n-pentyloxy.

"Halogen" is fluorine, chlorine, bromine and/or iodine.

When compounds of the invention comprise amino group(s), they may form salts with various acids such as inorganic acids like hydrochloride or sulfate, or an organic acid such as trifluoroacetic acid or acetic acid. When compounds of the invention comprise a carboxyl group(s), they can also form salts thereof with bases such as inorganic bases like sodium, potassium, calcium or ammonium salts, or such as organic bases like basic amino acid salts. Furthermore, a pharmaceutically acceptable salt is preferable.

Compounds of the invention can also be hydrates or solvates, such as with dioxane-water, which include such hydrates and solvates.

Compounds of the invention have at least one asymmetric atom. Thus, compounds of the invention are stereo-isomers or mixtures thereof, which are included in the invention. Configuration of αcarbon atom of the glutamyl moiety is preferably in a D-configuration, while configuration of the carbon atom conjugated with $-COOR_2$, in R_3, is preferably similar to L-amino acid configuration.

Compounds of the invention can be subsequently prepared by reacting a compound of the following formula or acid-addition salts thereof:

(II)

wherein:

R_2 and R_3 are the same as the above,

with a compound of the following formula or a derivative that is reactive at carboxyl group thereof:

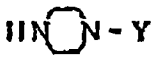
(III)

wherein:
R and R_1 are the same as the above,
optionally, followed by the removal of the protecting group from the resulting product, or optionally, by converting it to the salt thereof.

A compound in which R_1 of formula(III) is $-NH(CH_2)_m-W$ or -N N-Y can be prepared by reacting a compound of the following formula:

(IV)

(wherein:
X is halogen; R_7 is lower alkyl or benzyl; and R is the same as the above),
with a compound of the following formula:
$H_2N-(CH_2)_m-W$ (V-1)
(wherein:
n and W are the same as the above),
or



(V-2)

(wherein:
Y is the same as the above),
optionally, by removing the protecting group of carboxyl group.

The reaction of a compound of formula(II) with a compound of formula(III) may be carried out according to a common method known in the art(see, "Fundamental and Practice of Peptide Synthesis", Izumiya Nobuo, et al.,89-131p(Maruzen Company, Limited)).

When R_1 is a primary or secondary amino-group, or carboxyl group, a compound with a protection group thereof is preferably used. When a compound of formula(III) is

reacted with a compound of formula(II) with free carboxylic acid, a compound of formula(II) with protected -$COOR_2$ is preferably used. Furthermore, the reaction is carried out in the presence of a condensing agent, such as N,N-dicyclohexylcarbodiimide ,1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride salt, carbonylimidazole , diphenylphsophorylazide, cyanophosphate diethyl and the like. When carbodiimides are used as condensing agents, 1-hydroxybenzotriazole, N-hydroxysuccucinimide, N-hydroxy-5-norbornene-2,3-dicarboxy-imide may be added to the reaction mixture in order to prevent racemization.

Alternatively, the compound of formula(III) in a form of reactive derivative in terms of carboxyl group may be reacted with an amine compound of formula(II) in place of the above condensing agents.

Reactive derivatives in terms of carboxyl group of the compound of formula(III) include acid halides, acid azides, anhydrides of mixed acids, active esters, active amides and the like.

The above aforementioned reaction can be usually carried out in a solvent at -40-40°C. Solvents used therein include tetra-hydrofuran, dioxane, dimethylformamide, acetonitrile, ethanol, methanol and water, in which single or combined solvents may be used. When acids generate as by-products, or the compound of formula(II) used is an acid addition salt has a free carboxyl group, the reaction is preferably carried out in the presence of bases that function as acid acceptors. Such bases that can be used therein include alkaline hydroxide, such as sodium hydroxide, potassium hydroxide, alkaline carbonates and alkaline bicarbonates, such as $NaHCO_3$, Na_2CO_3, K_2CO_3 and the like, and organic bases, such as triethylamine, N-methylmorfoline, dicyclo-hexylamine, pyridine, 4-dimethyl-aminopyridine and the like.

In the above reaction, starting compounds with a protected amino group or protected carboxyl group can be

usually used in peptide synthesis. Any protecting group used in peptide synthesis can be used and preferably selected to be suitable for the object (see, "Fundamental and Practice of Peptide Synthesis", Izumiya Nobuo, et al.,148-152p(Maruzen Company, Limited)). Amino protecting groups include benzyloxycarbonyl, tert-butoxycarbonyl, 3-nitro-2-pyridine-sulfenyl and the like. After a reaction is completed, these protecting groups can be subsequently removed according to a known method.

Carboxyl protecting groups include methyl ester, ethyl ester, benzyl ester and the like. These protecting groups can be removed by hydrolysis using diluted alkaline (e.g.,1-2N NaOH or KOH). Benzyloxycarbonyl group or benzyl group of benzyl ester can be preferably removed by a catalytic reduction in the presence of palladium black, palladium-carbon or palladium-carbon/ammonium formate, or by the action of HBr/AcOH. A tert-butoxycarbonyl group or tert-butoxy group of tert-butoxy ester can be removed by the action of a strong acid, such as trifluoroacetic acid in a ice-bath or at room temperature.

Compounds of the invention prepared as the above can be also, optionally, converted to salts thereof described above using a usual method.

Compounds of the invention and salts thereof can be isolated or purified by known purification methods, such as extraction, condensation, neutralization, filtration, recrystallization, column chromatography, high-performance liquid chromatography or with ion exchange resin, optionally in combination.

Compounds of the invention and salts thereof are useful , based on ACE inhibitory activity thereof, as therapeutic agents for hypertension, ischemic cardiac insufficiency and other cardiovascular diseases.

Pharmacological action of compounds of the invention is described as below.

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In Vitro ACE Inhibitory Activity

ACE prepared from rabbit lung, synthetic substrate hippuryl-L-histidyl-L-leucine(5mM), sodium chloride(300mM) and phosphate buffer(100mM,pH8.3) were mixed to be the final volume of 0.300ml and reacted at 37°C for 30 minutes. 1N-Hydrochloride was added thereto to attenuate the reaction, followed by extraction of produced hippuric acid with ethyl acetate. After evaporation of ethyl acetate, distilled water was added and the absorbance thereof at 228 nm was measured, so that the amount of hippuric acid was determined by spectrophotometer(Hitachi 100-41).

The extent of ACE inhibition was calculated by comparing enzymatic activity with the test compounds to that with the control. The IC_{50}(the concentration at 50% inhibition) value was obtained from a dose-inhibition curve. The results are shown in table 1.

Table 1

Test compounds	ACE Inhibitory activity (IC ;M)
Compound of example 1	2.1×10^{-7}
Compound of example 2	4.6×10^{-7}
Compound of example 6	3.1×10^{-7}
Compound of example 7	7.8×10^{-8}
Compound of example 8	2.2×10^{-7}
Compound of example 9	1.5×10^{-8}
Compound of step 4 in example 1	3.3×10^{-7}

The following examples are intended to further illustrate the invention but are not limited to them.

EXAMPLES

Example 1

(2S,3aS,7aS)-1-[N-(2(R)-piperazinylpropionyl)-γ-D-glutamyl]octahydro-1H-indole-2-carboxylic acid

Step 1:

2(S)-Bromopropionic acid(28 g) was dissolved in toluene (50ml), followed by the addition of benzylalcohol(42 g) and para-toluenesulfonic acid(1.2 g) thereto, which was heated to reflux for 1 hour. Toluene was evaporated under vacuum and the residue was extracted with chloroform. The extract was washed with 5% $NaHCO_3$ and saturated NaCl, subsequently dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum to yield benzyl 2(S)-bromopropionate(17.0 g). bp.105-108 degree centigrade, $[\alpha]_D$ -6.5 degree(c=1/65,methanol).

Step 2:

1-Benzylpiperazine(1.6 g) and triethylamine(0.9 g) were dissolved in acetonitrile, to which benzyl(2)-bromo-propionate(2.2 g) was added and stirred to react at room temperature for 5 hours. After evaporation of acetonitrile under vacuum, the residue was subsequently extracted with chloroform. The extract was washed with 5% $NaHCO_3$ and saturated NaCl. The extract was then dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum to give the residue(2.5 g). Two grams thereof was dissolved in methanol(10 ml), to which 1N-NaOH(9 ml) was added and stirred to react at room temperature for 2 hours. After neutralization with diluted hydrochloride, methanol was evaporated and the residue was purified by column chromatography using a column(2.5ϕx40 cm) with CHP20P (Mitsubishi Chem. co. Ltd)(75-150μ)(0% → 50%, acetonitrile/water gradient) and concentrated by evaporation under vacuum to yield 2(R)-(4-benzyl-piperazinyl) propionic acid(1.0 g).

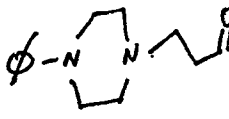
Step 3:

To a solution of methylene chloride containing N-Z-O^1-ethyl-D-glutamic acid(24.5 g), ethyl-(2S,3aS,7aS) octahydro-1H-indole-2-carboxylate hydrochloride salt(17.5 g) and tri-ethylamine(7.58 g), water soluble carbodiimide hydrochloride salt(15.8 g) was added, which was stirred overnight at room

temperature. The reaction mixture was then washed serially with saturated aqueous $NaHCO_3$, water, 10% hydrochloride and water, then dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum to give oily substance(34.1 g). Additionally, this substance was dissolved in ethanol(400 ml) followed by addition of 10% palladium-carbon(3 g) thereto and further ammonium formate (12 g) in three portions at room temperature with stirring. After 1 hour, the catalyst was filtrated off and the filtrate was acidified with hydrochloride and concentrated by evaporation. The residue was subsequently dissolved in water and washed with ethyl acetate. The water layer was alkalized with $NaHCO_3$ and extracted with methylene chloride. The combined organic layer was dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum to yield an oily substance, ethyl-(2S,3aS,7aS) -1-(O^1-ethyl-γ-D-glutamyl)octahydro-1H-indole-2-carboxylate(hereinafter referred to diester) (23.5 g). Next, 23 g of the above substance was dissolved in ethanol(150 ml), followed by the addition of 1N-NaOH(210 ml) thereto, which was stirred at room temperature for 5.5 hours. The reaction mixture was acidified with hydrochloride and concentrated under vacuum. The residual solution was purified by CHP20P column chromatography (0% → 30%, acetonitrile/water gradient).Fractions with high purity were concentrated by evaporation under vacuum to yield the purified substance(6.31 g). Fractions with low purity were also concentrated by evaporation under vacuum. The residue was dissolved in water and neutralized with $NaHCO_3$, which was also purified by CHP20P column chromatography(0% → 30%, acetonitrile/water gradient) to yield 8.70 g of the substance and, totally combined, 15.01 g of (2S,3aS,7aS)-1-(γ-D-glutamyl)octa-hydro-1H-indole-2-carboxylate(mp 191-192).

Step 4:

2(R)-(4-Benzylpiperazinyl)propionic acid(0.8 g) obtained in the step 2 was dissolved in chloroform(8 ml), to which N-

hydroxysuccinimide(0.4 g) and 1-ethyl- -
propyl)carbodiimide hydrochloride salt(d
to "water soluble carbodiimide hydroch )
were added and stirred to react at ro 3
hours. The reaction mixture was extra m
and the extract was washed with 5% N d
NaCl, dried over anhydrous sodium sufate and concentrated by evaporation to yield 2(R)-(4-Benzylpiperazinyl)propionic acid N-hydroxy-sccucinimide ester. This was dissolved in THF and was added dropwise at room temperature to THF-water mixed solvent in which (2S,3aS,7aS)-1-(γ-D-glutamyl)octa-hydro-1H-indole-2-carboxylate(1.0 g) and $NaHCO_3$(0.5 g) were dissolved. After the reaction mixture was stirred at room temperature for 3hours, THF was removed by evaporation under vacuum and the residue was then acidified with 10% citric acid and purified by CHP20P column chromatography (0%→40%, acetonitrile/water gradient), followed by concentrating the objective fraction by evaporation to yield (2S,3aS,7aS)-1-[N-(2(R)-(4-benzylpiperazinyl)propionyl]-7-D-glutamyl]octa-hydro-1H-indole-2-carboxylic acid(0.8 g)

$[\alpha]_D$-20.7° (c=0.49, 1N-NaOH),

For $C_{28}H_{40}N_4O_8 \cdot 2.5H_2O$,

Calculated: C,58.62; H,7.91; N,9.77.

Found: C,58.49; H,7.73; N, 9.82.

Step 5:

0.4 Grams thereof and 0.5 gram of formic acid were dissolved in methanol, to which palladium black(0.1 g) was added and heated to reflux for 4 hours. The catalyst was filtrated off therefrom and the filtrate was concentrated by evaporation under vacuum. The residue was then purified by CHP20P column chromatography (0% → 35%, acetonitrile/water gradient), followed by concentrating the objective fractions by evaporation to yield the objective compound(0.2 g).

$[\alpha]_D$-6.1° (c=0.71, H_2O),

For $C_{21}H_{34}N_4O_8 \cdot 1.25H_2O$,

Calculated: C,54.71; H,7.98; N,12.15.

Found: C,54.83; H,8.00; N,11.95.

Example 2

(2S,3aS,7aS)-1-[N-(2(R)-(n-butylamino)propionyl]-γ-D-glutamyl]octahydro-1H-indole-2-carboxylic acid

Step 1:

n-Butylamine(1.0 g) and triethylamine(1.4 g) were dissolved in chloroform, to which benzyl(2R)-bromopropionate(3.4 g) was added dropwise and stirred to react overnight at room temperature. The reaction mixture was subsequently extracted with chloroform and the organic layer was washed with 5% $NaHCO_3$ and saturated NaCl, then it was dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum to yield the residue(2.6 g). This was dissolved in methanol, to which 1N-NaOH(10 ml) was added and stirred to react at room temperature for 1 hours. After neutralization with diluted hydrochloride, the solvent was evaporated and the residue was purified by CHP20P column chromatography (0%→50%,acetonitrile/water gradient) and concentrated the objective fraction by evaporation under vacuum to yield 2(R)-n-butylaminopropionic acid(2.0 g).

Step 2:

1.0 g thereof was subsequently dissolved in 1N-NaOH(10 ml) and cooled in ice bath, to which benzyloxycarbonylchloride(1.2 g) was added dropwise and stirred to react at room temperature for 2 hours. The reaction mixture was acidified with diluted hydrochloride and extracted with ethyl acetate. The organic layer was then washed with water and saturated NaCl, dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum to yield 2(R)-(n-butylbenzyloxycarbonylamino) propionic acid(0.8 g).

Step 3:

This residue was dissolved in acetonitrile, to which N-hydroxy-succinimide(0.35 g) and water soluble carbodiimide

hydrochloride salt(0.6 g) were added and stirred to react overnight at room temperature. The reaction mixture was then extracted with chloroform and the organic layer was washed with 5% $NaHCO_3$ and saturated NaCl, dried over anhydrous sodium sulfate and concentrated by evaporation to yield 2(R)-(n-butylbenzyloxycarbonyl)propionic acid N-hydroxysccucinimide ester. Using this in place of 2(R)-(4-benzylpiperazinyl) propionic acid N-hydroxysccucinimide ester in step 4 of the Example 1, the same procedure as the step 5 was carried out to give the objective substance(0.39 g).

$[\alpha]_D$-30.3° (c=0.43, 1N-NaOH),

For $C_{20}H_{32}N_3O_6 \cdot 0.5H_2O$,

Calculated: C,57.26; H,7.93; N,10.02

Found: C,57.27; H,8.39; N, 9.58.

Example 3

(2S,3aS,7aS)-1-[N-(2(R)-(2-carboxyethylamino)propionyl-γ-D-glutamyl]octahydro-1H-indole-2-carboxylic acid

Step 1:

Methyl ester of β-alanine p-toluenesulfonate salt(3 g) and triethyl amine(2.2 g) were dissolved in acetonitrile, to which benzyl 2(S)-bromopropionate(2.6 g) was added dropwise and stirred to react at room temperature for 3.5 hours. The reaction mixture was extracted with chloroform. The organic layer was washed with 5% $NaHCO_3$ and saturated NaCl, dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum to yield 2(R)-(2-methoxycarbonylethylamino) propionic acid(1.4 g).

Step 2:

This was then dissolved in dioxane, to which di-tert-butyl-dicarbonate(1.2 g) was added dropwise and stirred to react at room temperature for 2 hours. The reaction mixture was extracted with chloroform. The organic layer was washed with 10% citric acid, 5% $NaHCO_3$ and saturated NaCl, dried over

anhydrous sodium sulfate and concentrated by evaporation under vacuum to yield the residue(1.4 g). This was then dissolved in methanol, to which palladium black(0.3 g) was added to carry out a catalytic reduction under atmospheric pressure at room temperature for 2 hours. The catalyst was subsequently filtrated off and the filtrate was concentrated by evaporation under vacuum to yield 2(R)-(2-methoxy-carbonylethyl-tert-butoxycarbonylamino)propionic acid (0.8 g).

Step 3:

This was then dissolved in chloroform, to which N-hydroxy-succinimide(0.35 g) and water soluble carbodiimide hydrochloride salt(0.6 g) were added and allowed to react at room temperature for 2 hours. The reaction mixture was extracted with chloroform and the extract was washed with 5% $NaHCO_3$ and saturated NaCl, dried over anhydrous sodium sulfate and concentrated by evaporation, which was dissolved in THF, followed by dropwise addition thereof to a mixed solvent of THF-water, dissolving (2S,3aS,7aS)-1-(-γ-D-glutamyl)octa-hydro-1H-indole-2-carboxylic acid(0.9 g) and $NaHCO_3$(0.5 g). After the reaction mixture was stirred to react at room temperature for 3hours, The solvent was removed by evaporation under vacuum and the residue was then acidified with 10% citric acid and purified by CHP20P column chromatography (0% → 70%, acetonitrile/water gradient), followed by concentrating the objective fraction by evaporation to yield (2S,3aS,7aS)-1-[N-[2(R)-(2-methoxy-carbonylethyl-tert-butoxyamino)-propionyl]-γ-D-glutamyl] octahydro-1H-indole-2-carboxylic acid(1.2 g)

Step 4:

This was dissolved in acetonitrile, to which 1N-NaOH(10 ml) was added and stirred to react at room temperature for 2 hours. The reaction mixture was acidified with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with saturated NaCl, dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum to give

the residue(0.8 g). This was dissolved in trifluoroacetic acid and stirred to react at room temperature for 1 hour. The reaction solution was evaporated under vacuum and the residue was then purified by CHP20P column chromatography (0% → 50%, acetonitrile/water gradient), followed by concentrating the objective fraction by evaporation to yield the objective substance(0.3 g).

$[\alpha]_D$-16.8°(c=0.97, 1N-NaOH),

For $C_{20}H_{31}N_3O_8 \cdot 1.5H_2O$,

Calculated: C,51.27; H,7.31; N,8.97

Found: C,51.31; H,7.40; N, 8.99.

Example 4

(2S,3aS,7aS)-1-[N-[2(R)-(3-carboxypropylamino)propionyl]-γ-D-glutamyl]octahydro-1H-indole-2-carboxylic acid

$[\alpha]_D$-27.1°(c=0.37, 1N-NaOH),

For $C_{21}H_{33}N_3O_8 \cdot 0.75H_2O$,

Calculated: C,53.78; H,7.41; N,8.96

Found: C,54.00; H,7.72; N, 9.06.

Example 5

(2S,3aS,7aS)-1-[N-[2(R)-(3-aminopropylamino)propionyl]-γ-D-glutamyl]octahydro-1H-indole-2-carboxylic acid

Step 1:

Trityl chloride(7.6 g) was added dropwise to trimethylenediamine(20g) cooled in an ice bath, which was stirred to react at 0-5°C for 30 minutes. The reaction mixture was diluted with water and acetonitrile, and purified by CHP20P column chromatography (0% → 100%, acetonitrile/water gradient), followed by concentrating the objective fraction by evaporation to yield the monotrityl derivative(7.3 g).

Step 2:

Using 5.1 g thereof in place of methyl ester of β-alanine p-toluenesulfonate salt used in the step 1 of the example 3,

the same procedure as the Step 2 and step 3 was carried out to yield (2S,3aS,7aS)-1-[N-[2(R)-(3-tritylaminopropyl-tert-butoxycarbonylamino)propionyl]-γ-D-glutamyl]octahydro-1H-indole-2-carboxylic acid(1.0 g). This was then dissolved in trifluoroacetic acid and stirred to react at room temperature for 1 hour. The solvent was evaporated under vacuum and the residue was neutralized with 1N-NaOH and, then purified by CHP20P column chromatography (0% → 30%, acetonitrile/water gradient), followed by concentrating the objective fraction by evaporation to yield the objective substance(0.12 g).

$[\alpha]_D$-22.1° (c=0.36, H_2O),

For $C_{20}H_{34}N_4O_8 \cdot 0.4H_2O \cdot 0.1CH_3CN$

Calculated: C,55.42; H,8.08; N,13.12

Found: C,55.33; H,8.57; N, 13.44

Example 6

N-[2(S)-(4-phenylpiperazinyl)propionyl]-γ-D-glutamyl-N-(p-methoxyphenyl)-L-alanine

Step 1:

p-Methoxyaniline(10.8 g) and N-methylmorpholine(7.4 g) were dissolved in chloroform(80 ml), to which 2(R)-bromopropionic acid(10.0 g) was added and heated to be refluxed for 3.5 hours. After cooling it, the crystalline N-(p-methoxyphenyl)-L-alanine(8.3 g) was obtained by filtration. 7.3g.thereof and thionyl chloride(6.7 g) were dissolved in ethanol(70ml), heated to be refluxed for 4 hours and concentrated under vacuum. The residue was extracted with chloroform. The organic layer was washed with 5% $NaHCO_3$ and water, dried over anhydrous sodium sulfate, concentrated by evaporation under vacuum to yeild N-(p-methoxyphenyl)-L-alanine ethyl ester(8.0 g).

Step 2:

The above obtained ester(7.9 g) and N-methylmorpholine(3.6 g) were dissolved in acetonitrile(60 ml), to which 3-(4(R)-3-benzyloxycarbonyl-5-oxooxazolizine-4-yl)propionic acid

chloride(11.0 g) was added and heated to be reflux for 5 hours. It was concentrated under vacuum and the residue was extracted with chloroform. The organic layer was washed serially with 10% citric acid, 5% $NaHCO_3$ and water, dried over anhydrous sodium sulfate and concentrated by evaporation. The residue was then purified by silica gel chromatography (eluent: chloroform) to yield N-[3-(4(R)-3-benzyloxy-carbonyl-5-oxooxazolizine-4-yl) propionyl]-N-(p-methoxyphenyl)-L-alanine ethyl ester(6.8 g). The above obtained ester(6.65 g) and sodium acetate(1.64 g) were dissolved in ethanol and stirred overnight at room temperature. The reaction mixture was concentrated by evaporation under vacuum. The residue was extracted with ethyl acetate. The organic layer was washed with 10% hydrochloride, 5% $NaHCO_3$, saturated NaCl and water, dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum. The residue was purified by CHP20P column chromatography (40% → 80%, acetonitrile /water gradient), followed by concentrating the objective fraction by evaporation to yield N-benzyloxy-carbonyl-O^1-ethyl-γ-D-glutamyl-N-(p-methoxy-phenyl)-L-alanine ethyl ester(3.5 g).

Step 3:

In ethanol(50 ml),2.7 g thereof was dissolved, to which 10% palladium carbon(0.5 g) was added and then ammonium formate(2 g) was further added with stirring. After 2hours, the catalyst used was filtrated off and the filtrate was evaporated by evaporation. The residue was extracted with methylene chloride and was washed with 5% $NaHCO_3$ and saturated NaCl, dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum to yield O^1-ethyl-γ-D-glutamyl-N-(p-methoxyphenyl)-L-alanine(1.83 g).

Step 4:

4-Phenylpiperazine(2.0 g) was dissolved in methylene chloride(20 ml), to which 2(R)-bromopropionic acid(5.3 g) was added, stirred overnight at room temperature, and concentrated by evaporation under vacuum. The residue was

purified by CHP20P column chromatography (0% → 30%, acetonitrile/water gradient), followed by concentrating the objective fraction by evaporation to yield 2(S)-(4-phenylpiperazinyl)propionic acid(2.0 g)(mp 200-209 °, decomposition). The above obtained acid (0.6 g), O^1-ethyl-γ-D-glutamyl-N-(p-methoxyphenyl)-L-alanine ethyl ester(0.8 g) and water soluble carbodiimide hydrochloride salt(0.46 g) were stirred in methylene chloride overnight at room temperature. The reaction mixture was then washed with 5% $NaHCO_3$ and water, dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum. The residue was purified by silica gel chromatography to yield N-[2(S)-(4-phenylpiperazinyl)propionyl]O^1-ethyl-γ-D-glutamyl-N-(p-methoxyphenyl)-L-alanine ethyl ester(0.54 g).

Step 5:

The above obtained ester(0.5 g) was dissolved in a mixed solvent of dioxane(20 ml)-water(10 ml), to which 1N-NaOH(3.6 ml) was added and stirred overnight at room temperature. It was neutralized with diluted hydrochloride, concentrated to a half volume, and purified by CHP20P column chromatography (0% → 50%, acetonitrile/water gradient), followed by concentrating the objective fraction by evaporation. The residue was dissolved in a mixed solvent of dioxane-water and freeze-dried to yield the objective substance(19 g) as white powder.

$[\alpha]_D$-0.8°(c=0. 6, ethanol),

For $C_{28}H_{38}N_4O_7 \cdot 1\ H_2O \cdot 0.2$ dioxane

Calculated: C,60.03; H,6.93; N,9.72

Found: C,59.86; H,6.71; N, 9.46

Example 7

N-[3-(4-phenylpiperazinyl)propionyl]-γ-D-glutamyl-N-cycloheptyl-DL-alanine

Step 1:

Cycloheptylamine(30 g) and N-methylmorpholine(10.7 g) were dissolved in acetonitrile(40 ml), to which 2(RS)-bromo-

propionic acid(19.2 g) was added and stirred at room temperature for 5 hours. It was concentrated under vacuum and the residue was extracted with ethyl acetate. The organic layer was washed serially with 5% $NaHCO_3$ and saturated NaCl, dried over anhydrous sodium sulfate and concentrated by evaporation. The residue was purified by silica gel chromatography(eluent: chloroform) to yield oily substance(22.0 g), which was dissolved with maleic acid(11.1 g) in ethanol(150 ml) and was concentrated by evaporation and the residue was recrystallized from isopropyl alcohol to yield ethyl ester of N-cycloheptyl-DL-alanine maleic acid salt(26.0 g)(mp 125-127°).

Step 2:

The above obtained ester(7.5 g) was dissolved in ethyl acetate. The organic layer was washed serially with saturated $NaHCO_3$ and saturated NaCl, dried over anhydrous sodium sulfate, concentrated by evaporation to yield ethyl ester of N-cycloheptyl-DL-alanine(4.4 g). With this(7.5 g) in place of ethyl ester of N-(p-methoxyphenyl)-L-alanine used in step 2 of Example 6, the same procedures as the step 2 and step 3 of Example 6 were carried out to yield O^1-ethyl-γ-D-glutamyl-N-cycloheptyl-DL-alanine ethyl ester(2.2 g).

Step 3:

4-Phenylbenzylpiperazine(10.1 g) and acrylonitrile(3.3 g) were reacted by stirring at 50°C for 2 hours, to which petroleum ether was added to allow crystallization. It was then recrystallized from 50% alcohol to yield 3-(4-phenyl-piperazinyl)propionitrile(10.4 g)(mp 74-75°). The above obtained nitrile(2.2 g) was then dissolved in conc. hydrochloride and heated to reflux overnight. The reaction mixture was concentrated by evaporation and the residue was purified by CHP20P column chromatography (0% → 50%, acetonitrile/water gradient), followed by concentrating the objective fraction by evaporation to yield 3-(4-

phenylpiperazinyl) propionic acid(1.9 g) (mp 186-187°,decomposition).

Step 4:

The above obtained acid(1.85 g) was dissolved in N,N-dimethylformamide(20 ml) and dimethylsulfoxide(20 ml) and cooled to -15°C with stirring. N-Methylmorpholine(0.55 ml), isobutyloxycarbonylchloride(0.71 ml) and a solution of O^1-ethyl-γ-D-glutamyl-N-cycloheptyl-DL-alanine ethyl ester(0.82 g) in THF(10 ml) obtained in the foregoing step 2 were serially added thereto and stirred at -15°C for 30 minutes. Subsequently, at room temperature for another 1 hour, and concentrated under vacuum. The residue was then extracted with ethyl acetate and the organic layer was washed with 5% $NaHCO_3$ and saturated NaCl, dried over anhydrous sodium sulfate and concentrated under vacuum to yield of N-[3-(4-phenyl-piperazinyl)propionyl]-O^1-ethyl-γ-D-glutamyl-N-cycloheptyl-DL-alanine ethyl ester(1.5 g).

Step 5:

With the above obtained ester(0.3g) in place of N-[2(S)-(4-phenylpiperazinyl)propionyl]O^1-ethyl-γ-D-glutamyl-N-(p-methoxyphenyl)-L-alanine ethyl ester used in the step 5 of example 6, the same procedure as the foregoing step was carried out to yield the objective substance(0.11 g).

$[\alpha]_D$+1.0°(c=0.2, ethanol),

For $C_{28}H_{42}N_2O_8 \cdot 1.8\ H_2O \cdot 0.4$dioxane

Calculated: C,59.42; H,8.22; N,9.36

Found: C,59.06; H,7.68; N, 9.53

Example 8

(2S,3aS,7aS)-1-[D-phenylalanyl-γ-D-glutamyl]octahydro-1H-indole-2-carboxylic acid

SStep 1

N-benzyloxycarbonyl-D-phenylalanine(4.8 g), N-hydroxy-5-norbornene-2,3-dicarboxyimide(2.9 g) and water souble carbodiimide hydrochloride salt(3.1 g) in methylene chloride were stirred to react at room temperature for 1.5 hours. The

reaction mixture was washed with saturated $NaHCO_3$ and 10% citric acid, dried over anhydrous sodium sulfate, and concentrated by evaporation to yield N-benzyloxycarbonyl-D-phenylalanine N-hydroxy-5-norbornene-2,3-dicarboxyimide ester (7.1 g), with which (2S,3aS,7aS)-1-(γ-D-glutamyl) octahydro-1H-indole-2-carboxylic acid(4.7 g) were dissolved in a mixed solvent of saturated $NaHCO_3$(200 ml) and THF(100 ml) and stirred overnight at room temperature. It was concentrated under vacuum and the residue was washed with ethyl acetate and the water layer was acidified with hydrochloride, followed by extraction thereof with chloroform. The combined organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum. The residue was purified by silica gel chromatography (eluent: chloroform: methanol: acetic acid =95:5:1) to yield (2S,3aS,7aS)-1-[N-benzyloxycarbonyl D-phenylalanyl-γ-D-glutamyl]octahydro-1H-indole-2-carboxylic acid(7.0 g)($[\alpha]_D$-12.9°(c=0.4,1N-NaOH).

Step 2

The obtained above substance (0.6 g) was dissolved in a solution of 25% anhydrous hydrobromide acetic acid(15 ml) and stirred at room temperature for 1 hour. The reaction mixture was concentrated under vacuum and the residue was purified by CHP20P column chromatography (0% → 30%, acetonitrile/water gradient) and the objective fraction was concentrated by evaporation under vacuum to yield the objective substance(0.4 g). $[\alpha]_D$-31.5°(c=0.40, 1N-NaOH),

For $C_{23}H_{31}N_3O_6 \cdot 6.5\ H_2O$

Calculated: C,60.78; H,7.10; N,9.25

Found: C,60.69; H,7.23; N, 9.30

Example 9

(2S,3aS,7aS)-1-[N-(6-aminohexanyl-γ-D-glutamyl]octahydro-1H-indole-2-carboxylic acid

Step 1

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6-(N-Benzyloxycarbonylamino)hexianoic acid(4.0 g), N-hydroxysuccinimide(1.74 g) and water soluble carbodiimide hydrochloride(2.9 g) in methylene chloride were stirred to react at room temperature for 5 hours. The reaction mixture was washed with saturated $NaHCO_3$ and 10% citric acid, dried over anhydrous sodium sulfate, and concentrated by evaporation to yield 6-(N-benzyloxycarbonylamino)hexianoic acid N-hydroxycussinimide ester(5.3 g). The above obtained substance(2.3 g), (2S,3aS,7aS)-1-(-γ-D-glutamyl) octa-hydro-1H-indole-2-carboxylic acid(2.0 g) and $NaHCO_3$(1.6 g) were dissolved in a mixed solvent of water-THF and stirred overnight at room temperature. It was concentrated under vacuum and the residue was washed with ethyl acetate and the water layer was acidified with hydrochloride, further followed by extraction thereof with methlenechloride. The organic layer was subsequently washed with water, dried over anhydrous sodium sulfate and concentrated by evaporation under vacuum. The residue was purified by silica gel chromatography (eluent: chloroform: methanol: acetic acid =95:5:1) to yield (2S,3aS,7aS)-1-[N-[6-(N-benzyloxyamino)hexanyl]-γ-D-glutamyl]octahydro-1H-indole-2-carboxylic acid(1.97 g)

$[\alpha]_D$-30.5°(c=0.52, 1N-NaOH).

Step 2

The above obtained substance(1.6 g) was dissolved in ethanol(20 ml). To this solution, 10% palladium carbon(0.5 g), and further ammonium formate(1.1 g) were successively added with stirring at room temperature. After 2 hours, a catalyst was filtrated off and the filtrates were concentrated by evaporation. The residue was further dissolved in water and the pH was adjusted to 3 with diluted hydrochloride, followed by purification by CHP20P column chromatography (0%→50%, acetonitrile/water gradient). The objective fraction was concentrated by evaporation under vacuum to yield the objective substance(0.45 g).

$[\alpha]_D$-38.2°(c=0.30, 1N-NaOH),

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For $C_{20}H_{33}N_3O_6 \cdot 0.5\ H_2O$

Calculated: C,57.13; H,8.15; N,9.99

Found: C,57.35; H,7.80; N,:10.35